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8/30/05 Michelle Hobson
Date Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:	Examiner: S. Zhou
WOLFFE et al.	Group Art Unit: 1631
Serial No.: 10/083,682	Confirmation No.: 1541
Filing Date: October 24, 2001	Customer No.: 20855
Title: **LIBRARIES OF REGULATORY SEQUENCES; METHODS OF MAKING AND USING SAME** (as amended)	

TRANSMITTAL LETTER

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

Sir:

Transmitted herewith for filing, please find the following documents:

x Appeal Brief (18 pages) with attached Claims Appendix (3 pages), Evidence Appendix (1 page) and Related Proceedings Appendix (34 pages)

x Return receipt postcard

The fee is calculated as follows:

	NO. OF CLAIMS	CLAIMS PREVIOUSLY PAID FOR	EXTRA CLAIMS	RATE	FEE
Total Claims	10	- 124	0	x $50.00	$0
Independent Claims	1	- 23	0	x $200.00	$0
Multiple dependent claims not previously presented, add $360.00					$0
Total Amendment Fee					$0
Appeal Brief Fee					$500.00
Small Entity Reduction (if applicable)					$250.00
TOTAL FEE DUE					$250.00

x A check in the amount of $250.00 is attached.

The Commissioner is hereby authorized to charge any appropriate fees under 37 C.F.R. §§1.16, 1.17, and 1.21 that may be required by this paper, and to credit any overpayment, to Deposit Account No. 18-1648.

Respectfully submitted,

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USSN 10/083,682
8325-0015.20
S15-US2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

A.P. Wolffe *et al.*

Application No.: 10/083,682

Filed: October 24, 2001

For: LIBRARIES OF REGULATORY SEQUENCES, METHODS OF MAKING AND USING SAME (as amended)

Examiner: S. Zhou

Group Art Unit: 1631

Confirmation No.: 1541

APPEAL BRIEF

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: A.P. Wolffe *et al.* Application No.: 10/083,682 Filed: October 24, 2001 For: LIBRARIES OF REGULATORY SEQUENCES, METHODS OF MAKING AND USING SAME (as amended)	Examiner: S. Zhou Group Art Unit: 1631 Confirmation No.: 1541

APPEAL BRIEF

Mail Stop Appeal Brief
Commissioner for Patents
Alexandria, VA 22313

Sir:

INTRODUCTION

Appellant submits one copy of this brief on appeal in accordance with Section 41.37 (69 Fed. Reg. 49962, Aug 2004). All claims were finally rejected under 35 U.S.C. §§112 (1st paragraph) and 103 in a Final Office Action mailed March 31, 2005. A Notice of Appeal was received by the USPTO on July 1, 2005, making a Brief on Appeal due September 1, 2005. Accordingly, this Appeal Brief is timely filed.

I. REAL PARTY IN INTEREST

Sangamo BioSciences, Inc., the assignee of record of the above-referenced patent application is the real party in interest in this matter.

II. RELATED APPEALS AND INTERFERENCES

Appellants have appealed the final rejection of all claims in parent application USSN 09/844,501. A copy of their Appeal Brief in that case is attached to the present Brief as the Related Proceedings Appendix. Appellant is not aware of any related interferences or judicial proceedings.

III. STATUS OF THE CLAIMS

Claims 66-71 and 125-128 are currently pending in the above-referenced case (hereinafter "the application"). The application was originally filed on October 24, 2001 with claims 1 to 124. Claims 1 to 124 were subject to a Restriction Requirement and, in an Amendment filed December 8, 2004, claims 1-65 and 72-124 were canceled; claims 66-71 were amended; and new claims 125-128 were presented. Claims 66-71 and 125-128 have not been amended since. Accordingly, claims 66-71 and 125-128 are pending as shown in the Claims Appendix. All pending claims remain rejected under 35 U.S.C. § 112, 1st paragraph (written description) and 35 U.S.C. § 102(b).

IV. STATUS OF THE AMENDMENTS

In response to the Examiner's Final Office Action mailed March 31, 2005, Appellant filed a Response on May 24, 2005. No amendments to the claims were made in that response. An Advisory Action was mailed on June 21, 2005 reiterating the rejections as set forth in the

Final Office Action. Thus, all claims remained rejected for the reasons set forth in the Final Office Action.

V. SUMMARY OF THE CLAIMED SUBJECT MATTER

The claimed subject matter relates to polynucleotides corresponding to accessible regions of cellular chromatin and libraries of these polynucleotides. Although previously it has been possible to identify accessible regions in cellular chromatin (*i.e.*, regions not packaged in a typical nucleosomal structure), the process of identification destroyed these sequences. Accordingly, it was previously not possible to isolate such accessible sequences. Thus, unlike libraries made from naked DNA, the presently claimed subject matter provides libraries that consist essentially of polynucleotides corresponding to accessible regions of cellular chromatin.

In particular, the claimed subject matter relates to polynucleotides that are members of a library of polynucleotides (page 8, lines 4-5; page 47, line 25 to page 55, line 20). The libraries comprise a vector and an insert in the vector (page 47, lines 30-33). The insert sequences consist essentially of accessible regions of cellular chromatin (page 47, lines 26-33). Moreover, the polynucleotide is obtained according to the method of: (a) contacting cellular chromatin with a probe, wherein reaction of the probe with cellular chromatin results in polynucleotide cleavage at accessible regions of cellular chromatin (page 4, line 29 to page 5, line 6); (b) deproteinizing the cleaved chromatin of step (a) (page 6, lines 14-17; page 51, lines 21-23; page 113, lines 11-14; page 123, lines 22-24); (c) digesting the deproteinized chromatin of step (b) with a nuclease to generate a collection of polynucleotide fragments (page 51, lines 21-23; page 123, lines 26-30); and (d) selectively cloning polynucleotide fragments comprising one end generated by probe cleavage (page 51, lines 31-34; page 124, lines 4-14).

The claims also relate to a library comprising a plurality of polynucleotides as described above (page 47, lines 26-29). The cellular chromatin from which the polynucleotides of the library are obtained may itself be obtained from cells at a particular stage of development and/or a particular tissue, for example diseased cells or infected cells (page 8, lines 11-14).

In addition, a probe that reacts with cellular chromatin, to obtain the polynucleotides as described herein, can be a nuclease (page 5, line 31), for example one or more restriction enzymes (page 6, line 15). Similarly, the deproteinized chromatin may digested with a restriction enzyme (page 6, lines 16-17).

VI. GROUNDS OF REJECTION

1. Claims 66-71 and 125-128 stand rejected under 35 U.S.C. 112, 1st paragraph as allegedly not adequately described by the specification as filed.

2. Claims 66-71 and 125-128 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by pages 177-183 of the Clontech Catalog (hereinafter "Clontech").

VII. ARGUMENTS

1. The Specification Describes the Claims on Appeal

Claims 66-71 and 125-128 remain rejected under 35 U.S.C. § 112, first paragraph as allegedly not described by the specification as filed. In support of the rejection, the Advisory Action stated (pages 3-4):

> While it is true that the claims and disclosure in Regents of the Univ. Calif. v. Eli Lilly are different from those in the instant application, it is in the same state of art: novel nucleic acids and/or polypeptides. Moreover, the fact patterns in both cases are similar in that they both claim nucleic acids and/or polypeptides whose sequences are not disclosed. Thus, the court decision in Regents of the Univ. Calif. v. Eli Lilly applies to this case. The sequences of SEQ ID NOs:10, 11, and 12 are not representative of all the species of [the] claimed genus.

Thus, it was alleged that, as in the *Eli Lilly* case, the specification at hand fails to describe sufficient representative species to describe the claimed genus of libraries.

Appellant submits that there is ample description in the specification regarding libraries and polynucleotides as claimed and there is no requirement that specific sequences exemplified

in the specification be recited in the claims. As such, the written description requirement of 35 U.S.C. § 112, first paragraph has been satisfied.

(a) The Holding in *Eli Lilly* Is Not Relevant To The Case At Hand

For the reasons of record, the Office's reliance of *Regents of the Univ. Calif. v. Eli Lilly* remains misplaced. The written description requirement of § 112 is highly fact-dependent and, contrary to the statements in the Advisory Action, the claims, disclosure and state of the art in *Eli Lilly* are entirely different from those in the case at hand.

First, the claims in the pending case are product-by-process claims whereas the claims in *Eli Lilly* were directed to polynucleotides *per se*. Further, the disclosure in Appellant's case includes actual exemplification of the products (polynucleotides such as SEQ ID NOs:10, 11 and 12) obtained by the recited process steps. *See, e.g.,* Example 15, pp. 123-126. In contrast, the disclosure in the *Eli Lilly* case did not describe either the isolation or the sequence of the claimed product (human insulin cDNA).[1] Finally, the relevant state of the art in each case is entirely different -- making libraries of non-coding sequences (accessible regions) prepared by recited, described and exemplified method steps (Appellant) versus obtaining novel coding sequences (*Eli Lilly*).

Thus, because the claims, disclosure and relevant state of the art in *Eli Lilly* are entirely different from the claims, disclosure and relevant state of the art case on appeal, the Federal Circuit's holding in *Eli Lilly* is not relevant to the question of whether the written description requirement is fulfilled for the subject matter of the appealed claims.

[1] Therefore, the Examiner is inaccurate in asserting that this case is like *Eli Lilly* in that no nucleic acid sequences are disclosed. *See,* page 2 of the Advisory Action, reproduced above. In fact, the specification clearly discloses exemplary polynucleotide sequences of accessible regions in the Examples as filed.

(b) The Claims Are Drawn to Polynucleotides And Libraries Fully Described By The Specification As Filed

It is well-settled law that the fundamental factual inquiry in written description is whether the specification conveys with reasonable clarity to those skilled in the art that, as of the filing date sought, applicant was in possession of the invention as now claimed. *See, e.g., Vas Cath, Inc. v. Mahurkar*, 935 F.2d 1557, 19 USPQ2d 1111. Determining whether the written description requirement is satisfied is a question of fact and the burden is on the Examiner to provide evidence as to why a skilled artisan would not have recognized that the applicant was in possession of the claimed invention at the time of filing. *Vas Cath, Inc. v. Mahurkar*, 19 USPQ2d 1111 (Fed. Cir. 1991); *In re Wertheim*, 191 USPQ 90 (CCPA 1976). It is not necessary that the application describe the claimed invention in *ipsis verba*. Rather, all that is required is that the specification reasonably convey possession. *See, e.g., In re Lukach*, 169 USPQ 795, 796 (CCPA 1971).

The Patent Office Guidelines are in accord and stress not only proper claim construction prior to analysis, but also indicate that the written description requirement is highly fact-dependent and there is a strong presumption that an adequate written description of the claimed invention is present at the time of filing (Final Examiner Guidelines on Written Description, 66 Fed. Reg. 1099).

Thus, any written description inquiry must begin with proper claim construction. Here, the claims on appeal are <u>not</u> drawn to any and all polynucleotides having any sequence. In fact, the genus encompassed by the claims on appeal is nowhere near as broad as that painted by the Examiner. It is simply those sequences, in the chromatin of a given cell, that are accessible to a probe.

Even claims 66 and 125-128, which as noted in the Advisory Action, are directed to "a" polynucleotide, clearly indicate that the claimed polynucleotide is a member of a library of accessible regions of cellular chromatin. By definition, a library contains a number of different

sequences, and, as is well known to those of skill in the art of molecular biology, it is impossible to predict the identity of the sequences that will be obtained after construction of a library.[2]

Thus, conception of the claimed polynucleotides libraries does not, indeed cannot, require description of the nucleotide sequence of every member of the library. Satisfaction of the written description does not require a showing that the skilled artisan can predict *a priori* each and every nucleotide sequence falling within the scope of the claims, but, rather, a demonstration that one of skill in the art would be aware an applicant was in possession of methods for making libraries of accessible regions of cellular chromatin, and of the libraries obtained through the practice of those methods. Here, the skilled artisan, having followed the teaching of the specification, would have no doubt that Appellant was in possession of a sequence corresponding to an accessible region of cellular chromatin, or to a library of such sequences.

Furthermore, the claimed polynucleotides must not only correspond to accessible regions of cellular chromatin, they must have been obtained using the particularly specified method, which results in isolation of such accessible regions. Thus, the claims at issue are product-by-process claims and, as such, are subject to a written description test much different from that used for product claims (including those in *Eli Lilly*) (*see,* M.P.E.P. § 2163):

> ...where the process has actually been used to produce the product, the written description requirement for a product-by-process claim is clearly satisfied.

With respect to claims 66 and 125-128, the product is a polynucleotide which is a member of a library. Example 15 describes three such products (SEQ ID NOs: 10, 11 and 12) obtained from two different libraries. Thus, when the product-by-process claims on appeal are properly construed, it is plain that they are drawn to a genus of polynucleotide library members that is more than adequately described by the specification as filed. In addition to fully describing the claimed polynucleotides and libraries, Appellant's specification also describes how the process steps recited in the claims have actually been used to produce these

[2] This fact points to yet another reason why the decision in *Eli Lilly* is not applicable to the present case: in *Eli Lilly*, it was possible for the inventors to identify the sequence they were trying to claim.

polynucleotides and libraries.[3] Thus, the rejection of the pending product-by-process claims is improper.

(c) Disclosure Of A Single Species Can Satisfy The Written Description Requirement

As noted above, the Examiner also errs in asserting that insufficient representative species are disclosed. As noted above, sequences are in fact exemplified and these exemplified sequences are more than representative of the genus encompassed by the claims. The flexibility and wide applicability of the claimed compositions should not be used as a basis for asserting that they are incompletely described; and any requirement for Appellants to actually provide more examples of such polynucleotides than already described is unnecessary for compliance with the written description requirement; moreover, limitation of the claimed subject matter to the exemplary sequences disclosed in the specification would prevent Appellants from claiming what they believe to be their invention.

Indeed, it is well settled that description of a single species can provide an adequate description, even for a broad genus. Thus, Appellant's disclosure is sufficient to establish that Appellant was in possession of the claimed subject matter at the time of filing.

In this regard, the PTO guidelines on written description include various Examples that establish that disclosure of a single species can more than adequately describe a genus. These Examples were favorably commented on by the Federal Circuit in *Enzo Biochem Inc. v. Gen-Probe Inc.*, 323 F.3d (Fed. Cir. 2002). In particular, Example 10, entitled "Process claim" states the following (underlining added):

> **Claim**:
> Claim 1: A process of producing an isolated polynucleotide comprising hybridizing SEQ ID NO:10 to genomic DNA in 6XSSC and 65°C and isolating the DNA polynucleotide detected with SEQ ID NO:10.

[3] Although the sequences of a large number of additional members of the libraries described in Example 15 were obtained by the inventors, their disclosure would have added nothing to the application that was not already provided by the sequences which were disclosed.

Claim 2: An isolated DNA that hybridizes with SEQ ID NO:10.

Analysis:

... The specification presents an example where a single species has been reduced to practice, i.e., isolation of SEQ ID NO:11 based on hybridization with SEQ ID NO:10. Therefore the disclosed species within the genus has been adequately described. Now turning to the genus analysis, the art indicates that there is no substantial variation within the genus because of the stringency of hybridization conditions which yields structurally similar molecules. The single disclosed species is representative of the genus because reduction to practice of this species, considered along with the defined hybridization conditions and the level of skill and knowledge in the art, are sufficient to allow the skilled artisan to recognize that applicant was in possession of the necessary common attributes or features of the elements possessed the members of the genus. ...

Conclusion:

Claim 1 is adequately described. ...

Note: Applicant may overcome the written description rejection of the product by, for example, substituting claim 2 with a product-by-process claim such as the one below.

Claim 2. *The isolated DNA polynucleotide prepared according to the process of claim 1.*

The written description inquiry for the product-by-process claims on appeal is highly analogous to that of Example 10, particularly the Patent Office's indication that product claim 2 of Example 10 would be adequately described if rewritten in product-by-process format to include novel process steps. In the case on appeal, all of the claims are already in product-by-process form, using a novel process that allows for the isolation of sequences corresponding to accessible regions from cellular chromatin. Further, the art indicates that it was conventional at the time of filing to make libraries by inserting polynucleotide sequences into a vector backbone. In other words, the specification's clear description (and indeed exemplification) of the unconventional elements of the claimed subject matter, namely the novel process steps, is more

than ample to indicate satisfaction of the written description requirement by evincing possession at the time of filing.

Moreover, a person of skill in the art would not expect substantial variation among species encompassed within the scope of the claims because of the requirement in the claims that the library inserts consist essentially of accessible regions and because of the requirement that specific process steps be used to obtain these polynucleotides and libraries. Therefore, it would be expected that the claimed (novel) process steps would yield polynucleotides corresponding to accessible regions. Accordingly, a representative number of species is disclosed, in view of the novelty of the process steps and the high level of skill and knowledge in the art.

Like Example 10, Example 18 of the PTO Guidelines, entitled "Process claim where the novelty is in the method steps" also illustrates a fact pattern that is highly instructive in the pending case (Example 18, underlining added):

> **Specification**: The specification teaches a method for producing proteins from mitochondria from the fungus *Neurospora crassa*. In the method, mitochondria are isolated from this fungus and transformed with a mitochondrial expression vector which comprises a nucleic acid encoding a protein of interest. The protein is subsequently expressed, the mitochondria [ ] lysed, and the protein is isolated. The specification exemplifies the expression of β-galactosidase using the claimed methods using a cytochrome oxidase promoter.
>
> **Claim**:
> 1. A method of producing a protein of interest comprising:
> obtaining *Neurospora crassa* mitochondria,
> transforming said mitochondria with an expression vector comrpising a nucleic acid that encodes said protein of interest,
> expressing said protein in said mitochondria, and
> recovering said protein of interest.
>
> **Analysis**:
> ...A search of the prior art reveals that the claimed method of expression in *Neurospora crassa* is novel and unobvious. ...
>
> There is actual reduction to practice of a single embodiment, i.e., the expression of β-galactosidase.

> The art indicates that there is no substantial variation within the genus because there are limited number of ways to practice the process steps of the claimed invention.
>
> The single embodiment is representative of the genus based on the disclosure ... considered along with the level of skill and knowledge in the gene expression art. One of skill in the art would recognize that applicant was in possession of all of the various expression methods necessary to practice the claimed invention.
>
> **Conclusion**: The claimed invention is adequately described.

As with Example 10, the claim, analysis and conclusion set forth in PTO Example 18 are also directly relevant and analogous to the written description analysis in the pending case. In particular, the pending product-by-process claims are analogous to the process claim presented in PTO Example 18 in that they recite a novel process (method of expression in Example 18 and method of isolating accessible regions in the claims on appeal) and include actual reduction to practice.

Thus, actual reduction to practice of a single disclosed species is more than sufficient to satisfy the written description requirement in the case at hand because, as in PTO Examples 10 and 18, the process steps are novel. Put another way, a person having ordinary skill in the art would conclude that applicant was in possession of all of the various polynucleotides and libraries obtained from the methods recited in the claims.

There is absolutely no requirement that Appellant describe each and every polynucleotide member of a library as claimed. Rather, the test is whether the specification contains sufficient disclosure to convey possession of the claimed subject matter. For the reasons of record, reiterated herein, the specification as filed, in view of the state of the art, more than adequately describes the claimed polynucleotides and libraries.

2. Anticipation Has Not Been Established

In the Advisory Action, the rejection of all pending claims are allegedly anticipated by the Clontech catalog was reiterated (Advisory Action, page 2):

> In regard to the rejection of claims 66-71 and 125-128 under 35 U.S.C. § 102(b) as being anticipated by Clontech ..., applicants' argument is on the ground that member of the Clontech libraries do not comprise an insert that consists essentially of accessible regions. This not deemed persuasive because as set forth in the previous Office Action, the phrase "consists essentially of" is interpreted as being open to unlisted ingredients. In this case, it is open to nucleotides from inaccessible regions. As also set forth in the previous Office Action, the Clontech libraries are made by a method involving digesting the whole genomes of the chromatin of different organisms ... . It would be readily apparent to one of skill in the art that the libraries produced by such a method inherently comprise clones that have an insert that either consists of polynucleotides from regions of cellular chromatin that are accessible .... the use of the phrase "consist essentially of" in the claims indicates that [the] claims are open to such [inaccessible] polynucleotides.

In other words, the Examiner asserts that Clontech's libraries, which are made from a completely different process than that recited in the pending claims, would somehow inherently produce polynucleotides that correspond to accessible regions and libraries that consist essentially of such polynucleotides. Such assertions are not supported by any evidence and are untenable.

(a) The Transition Phrase "Consisting Essentially Of" Cannot Be Interpreted As Being Open to Any Unlisted Ingredient

The assertion that the transitional phrase "consisting essentially of" renders the claims open to the inclusion of inaccessible regions is contrary to basic tenets of claim drafting and claim construction. See, for example, MPEP 2111.03:

> The transitional phrase "consisting essentially of" limits the scope of a claim to the specified materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention. . . . For the purposes of searching for and applying prior art under 35 U.S.C. 102 and 103,

> absent a clear indication in the specification or claims of what the basic and novel characteristics actually are, "consisting essentially of" will be construed as equivalent to "comprising." (citations omitted, emphasis in original)

In the case at hand, the basic and novel characteristics of the claimed polynucleotides and libraries are that they arise from, and correspond to, accessible regions of cellular chromatin. These basic and novel characteristics are recited in independent claim 66, both in the preamble and in step (a), in which it is recited that cellular chromatin (not naked cellular DNA) is contacted with a probe.

Thus it is clear that the claimed subject matter is directed to polynucleotides that consist essentially of accessible regions of cellular chromatin (and libraries of these polynucleotides). Clearly, the phrase "consists essentially of" in the present context would not read on polynucleotides corresponding to inaccessible regions, as such polynucleotides are not "unspecified" but would materially affect the basic and novel characteristic of the claimed polynucleotides.

Thus, the Examiner errs in concluding that the claims read on polynucleotides corresponding to both accessible and inaccessible regions. The language of the claims themselves indicates that polynucleotides corresponding to inaccessible regions are not the claimed subject matter.

(b) Clontech Does Not Expressly Or Inherently Disclose Each And Every Element Of the Pending Claims

It is also error to assert that Clontech discloses, expressly or inherently, the elements of the pending claims.

As previously pointed out by Appellants, the Clontech catalogue discloses nothing more than genomic libraries obtained by digestion of naked DNA.[4] Although the Examiner has

[4] See, *e.g.*, Responses dated December 8, 2004 and May 24, 2005

asserted that the Clontech libraries are obtained by digestion of chromatin[5], he has provided absolutely no evidence to support such an allegation.

Plainly, Clontech does not expressly describe or demonstrate (1) a polynucleotide member of a library including inserts consisting essentially of accessible regions of cellular chromatin; (2) contacting cellular chromatin with a probe that cleaves the cellular chromatin at accessible regions of cellular chromatin; and/or (3) deproteinizing the cleaved cellular chromatin -- essential elements of each and every pending claim.[6] Rather, Clontech describes a library (and library members) made from naked DNA, a substance that is very different from cellular chromatin. By no stretch of the imagination could digestion of naked DNA, under any circumstances, possibly produce a library of polynucleotides consisting essentially of accessible regions, as claimed. Rather, it produces a collection of polynucleotides representative of the entire genome and therefore consisting of both accessible and inaccessible sequences. Furthermore, inasmuch as naked DNA is devoid of associated proteins (*i.e.*, it is not chromatin), Clontech cannot possibly teach the deproteinizing step required by the claims on appeal. Accordingly, Clontech fails to expressly teach each and every claim element and, therefore, cannot expressly anticipate any of the appealed claims.

Nor does the Clontech catalog inherently anticipate the claims on appeal. As previously noted, inherency cannot be established by probabilities or possibilities, and the burden is on the Office to provide factual and technical grounds establishing that the inherent feature necessarily flows from the teachings of the reference *See, e.g., Continental Ca Co. USA, Inc. v. Monsanto Co.* 20 USPQ2d 1746, 1749 (Fed. Cir. 1987). This is true with regard to structural, functional and process limitations. Indeed, as the Board of Patent Appeals and Interferences and Federal Circuit have repeatedly established, "the examiner must provide some evidence or scientific reasoning to establish the reasonableness of the examiner's belief that the functional limitation is

[5] See, *e.g.*, Office Action dated September 9, 2004 at page 12; Office Action dated March 31, 2005 at page 8 and Advisory Action dated June 21, 2005 at page 2

[6] In fact, the Examiner acknowledges Clontech's failure to expressly disclose the claimed subject matter inasmuch as the rejection is based solely on alleged inherent disclosure.

an inherent characteristic" of the reference. *Ex parte Skinner,* 2 USPQ2d 1788 (BPAI 1986), emphasis added.

The Office has provided no such evidence or reasoning, but, instead, has merely asserted that the cited reference, disclosing a genomic library, inherently discloses the particularly claimed subject matter.

In reality, as noted in the record and above, digestion of naked DNA (as described in Clontech) will necessarily result in a collection (library) of DNA fragments that include both accessible and nonaccessible regions, as there are no proteins in naked DNA to protect any sequences from digestion. By contrast, the claimed libraries are composed of fragments from accessible regions, as they are made from digestion of cellular chromatin, in which chromosomal proteins protect non-accessible regions from digestion. The recited process steps thus impart structural and functional characteristics that fully distinguish the claimed polynucleotides from those of Clontech. Plainly, because a library consisting essentially of accessible regions and prepared from cellular chromatin as claimed is **not** an inherent feature of Clontech's libraries, the Clontech catalog does not anticipate the pending claims.

Thus, Clontech fails to describe, expressly or inherently, polynucleotides and libraries as claimed. Therefore, Appellant submits that the rejection cannot be sustained and should be withdrawn.

CONCLUSION

For the reasons stated above, Appellant respectfully submits that the specification adequately describes the pending claims and that the pending claims are patentable over the art cited by the Examiner. Accordingly, Appellant requests that the rejections of the claims on appeal be reversed, and that the application be remanded to the Examiner so that the appealed claims can proceed to allowance.

Respectfully submitted,

Date: August 30, 2005

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CLAIMS APPENDIX

CLAIMS ON APPEAL

1 to 65. (canceled).

66. (previously presented) A polynucleotide, wherein the polynucleotide is a member of a library of polynucleotides, the members of the library comprising a vector and an insert, wherein the insert sequences consist essentially of accessible regions of cellular chromatin, wherein the library is obtained according to the method of:

(a) contacting cellular chromatin with a probe, wherein reaction of the probe with cellular chromatin results in polynucleotide cleavage at accessible regions of cellular chromatin;

(b) deproteinizing the cleaved chromatin of step (a);

(c) digesting the deproteinized chromatin of step (b) with a nuclease to generate a collection of polynucleotide fragments; and

(d) selectively cloning polynucleotide fragments comprising one end generated by probe cleavage.

67. (previously presented) A library comprising a plurality of polynucleotides according to claim 66.

68. (previously presented) The library of claim 67, wherein the cellular chromatin is obtained from cells at a particular stage of development.

69. (previously presented) The library of claim 67, wherein the cellular chromatin is obtained from cells of a particular tissue.

70. (previously presented) The library of claim 67, wherein the cellular chromatin is obtained from diseased cells.

71. (previously presented) The library of claim 67, wherein the cellular chromatin is obtained from infected cells.

72 to 124. (canceled).

125. (previously presented): The polynucleotide of claim 66, wherein, in step (a), the probe is a nuclease.

126. (previously presented): The polynucleotide of claim 125, wherein the nuclease is a restriction enzyme.

127. (previously presented): The polynucleotide of claim 126, wherein the probe comprises a plurality of restriction enzymes.

128. (previously presented): The polynucleotide of claim 66, wherein, in step (c), the nuclease is a restriction enzyme.

EVIDENCE APPENDIX

No documents are submitted with the Evidence Appendix.

RELATED PROCEEDINGS APPENDIX

As noted above on page 2 of this Brief on Appeal and pursuant to 37 C.F.R. § 41.37(c)(i) and (c)(x), Appellant has filed an Appeal Brief in USSN 09/844,501, which is the parent of the instant application. Accordingly, a copy of this Appeal Brief and accompanying documents is submitted with this Appendix.

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VOLUME 2

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INTRODUCTION

The usual approach to isolating a recombinant DNA clone encoding a particular gene or mRNA sequence is to screen a recombinant DNA library. As described in Chapter 5, a recombinant DNA library consists of a large number of recombinant DNA clones, each one of which contains a different segment of foreign DNA. Since only a few of the thousands of clones in the library encode the desired nucleic acid sequence, the investigator must devise a procedure for identifying the desired clones. The optimal procedure for isolating the desired clone involves a positive selection for a particular nucleic acid sequence. If the desired gene confers a phenotype that can be selected in bacteria, then only the desired clone will grow under selective conditions, and it can be isolated in a rapid, effortless fashion. However, most eukaryotic genes and even many bacterial sequences do not encode a gene with a selectable function. Clones encoding nonselectable sequences are identified by screening libraries: the desired clone is identified either because it hybridizes to a nucleic acid probe or because it expresses a segment of protein that can be recognized by an antibody.

Screening libraries involves the development of a rapid assay to determine whether a particular clone contains the desired nucleic acid sequence. This assay is used first to identify the recombinant DNA clone in the library and then to purify the clone (see Fig. 6.0.1). Normally, this screening procedure is performed on bacterial colonies containing plasmids or cosmids or on bacteriophage plaques. To test a large number of clones at one time, the library is spread out on agarose plates (*UNIT 6.1*), then the clones are transferred to filter membranes (*UNIT 6.2*). The clones can be simultaneously hybridized to a particular probe (*UNITS 6.3* and *6.4*) or bound to an antibody (*UNIT 6.7*). When the desired clone is first identified, it is usually found among many undesirable clones; an important feature of library screening is the isolation of the desired clones (*UNITS 6.5* and *6.6*). Another method for identifying the desired clone involves hybrid selection (*UNIT 6.8*), a procedure in which the clone is used to select its mRNA. This mRNA is characterized by its translation into the desired protein.

To screen a DNA library, one must first devise the screening procedure. The next important choice is the selection of a recombinant DNA library. When choosing which library to screen the investigator should consider whether he or she wants to isolate clones encoding the gene or the mRNA sequence. cDNA clones will encode the mRNA

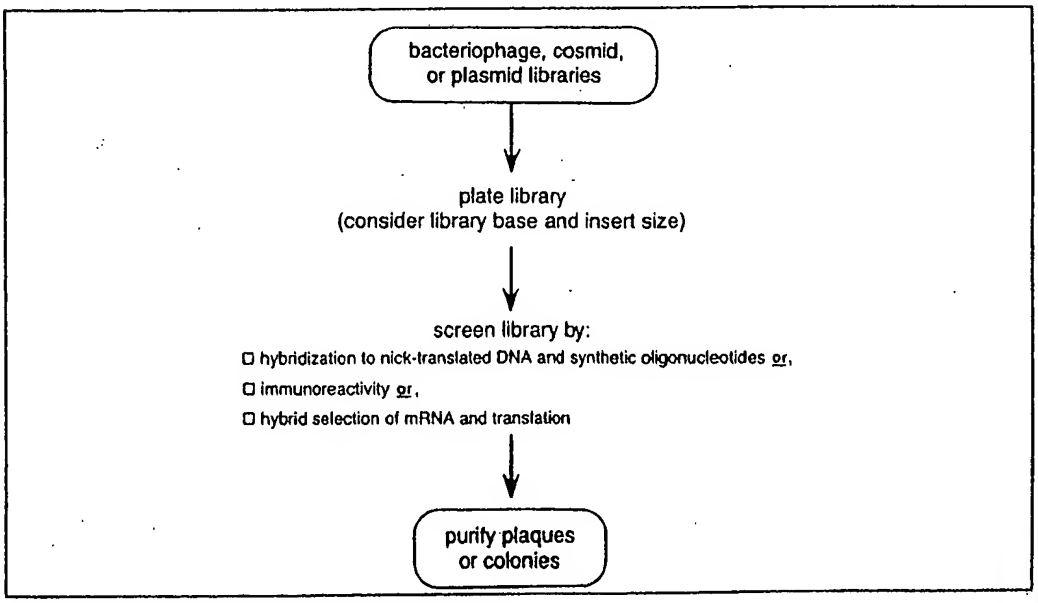

Figure 6.0.1 Flow chart for screening libraries.

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Guide to Molecular Cloning Techniques

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plaques *in situ* to replace those that have been removed during the preparation of filters or to increase subsequent hybridization signals.

1. Prepare an overnight culture of plating bacteria (this volume [13 or 17]). You will need 400 ml of culture for 20 filters.
2. Collect bacteria by centrifugation and resuspend them in an equal volume of fresh LB + 10 m*M* $MgSO_4$. General methods for handling λ are found in this volume [13].
3. Label the filters and mark them asymmetrically, with a black ballpoint pen, on the side that will be in contact with the plaques.
4. Dip the filters in the bacterial cell suspension and allow them to air dry briefly.
5. Lay the filters on the surface of the plates containing plaques. Transfer the orientation marks to the agar plate. The techniques in step 4 of the alternate procedure can be employed.
6. Prepare additional filter copies, if desired. Be sure to transfer orientation marks from agar to filter. A light box is a useful aid here.
7. Lay copy filters, phage plaque side up, on fresh LB + Mg^{2+} plates and incubate, inverted, at 37° overnight.

During the overnight growth at 37° the plaques infect the growing *E. coli*, leading to a substantial amplification of phage DNA. After this amplification, it is usually not necessary to hybridize two sets of filters to avoid false positives.

8. Remove the filters from the plates, air dry for at least an hour and process filters as in step 16, omitting the 10% SDS treatment as described above. Store plates, inverted, sealed in Parafilm at 4°. The filters are ready for prehybridization (this volume [45]).

[45] Screening Colonies or Plaques with Radioactive Nucleic Acid Probes

By GEOFFREY M. WAHL and SHELBY L. BERGER

Colony or plaque hybridization is a technique for screening replicated material *in situ* on filters with labeled probes.[1–5] The probes most com-

[1] M. Grunstein and D. S. Hogness, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3961 (1975).
[2] M. Grunstein and J. Wallis, this series, Vol. 68, p. 379.
[3] W. D. Benton and R. W. Davis, *Science* **196**, 180 (1978).
[4] D. Hanahan and M. Meselson, *Gene* **10**, 63 (1980).
[5] D. Hanahan and M. Meselson, this series, Vol. 100, p. 333.

monly used are nucleic acids or antibodies. Here we will describe techniques for using nucleic acids to analyze libraries generated in either phages or plasmids. The use of antibodies for screening libraries can also be found in this volume [50, 51].

A library is a mixture of clones constructed by inserting either cDNA or fragments of genomic DNA into a suitable vector. The term *library* implies the existence of large numbers of different recombinants, only one or a few of which are of immediate interest to the investigator. The desired clone is located by performing the following steps: (1) transfected bacteria or phage are grown on master plates (or filters) and replica plated; (2) the original plates called *master plates* are preserved while the replicas, hereafter called *filters*, are processed; (3) phage are disrupted or bacteria are lysed *in situ* on filters; (4) DNA is bound to the filter while RNA is hydrolyzed; (5) the resulting partially denatured DNA is hybridized to sequences able to bind specifically to the desired insertions. (6) Because the configuration of DNA on the filter replicas matches the configuration of live bacteria or phage on the master plates, DNA on replicas which binds to the probe (so-called positive signals) can direct the investigator to the bacterial colony or phage plaque from which the DNA was derived; (7) the positive colony or plaque is then purified and grown in quantity for further analysis.

Chapters [44] and [18] describe steps 1–4 for plasmid or λ libraries and cosmid libraries, respectively. Here we will focus on steps 5–7.

Colony hybridization is a rapid but inexact procedure aimed at calling attention to clones worthy of serious consideration. Falsely positive clones are therefore not uncommon. To some extent these can be reduced by the following: (1) use both negative control filters and, if possible, positive control filters; (2) screen duplicate filters of each master plate; and (3) prepare probes carefully.

To satisfy the requirements of point 1, it is advisable to include clones containing the vector without an insert or containing an irrelevant insert. The latter is particularly important when fragments bearing homopolymer "tails," usually composed of dG on one strand and dC on the other, are screened; the GC-rich regions on either end of the insert can hybridize to GC-rich probes and cause spurious positive signals. Thus, the use of known negative recombinants acts as a means for detecting unwanted cross-hybridization of the probe to vector and host DNA (which are also present) and also serves to establish the intensity of a background signal, one that should be ignored. Since intensities are relative, a genuine positive signal is needed for comparison. If there are no known positive clones, one can always clone the probe itself and create a positive recombinant. Such engineered positive colonies or plaques are rarely perfect

The claims, as currently written, are drawn to library or libraries of polynucleotides comprising polynucleotides corresponding to the accessible regions of cellular chromatin obtained by the method of claim 3. Each of these claims is directed to a genus comprising any library of polynucleotides comprising polynucleotides corresponding to the accessible regions of cellular chromatin obtained by the method of claim 3. Note that absent an explicit definition in the specification of the term, a library is interpreted as "an unordered collection of clones (i.e., cloned DNA from a particular organism)" (see Biotech Life Science Dictionary, URL: http://biotech.icmb.utexas.edu/search/dict-search.phtml?title=library). Thus the number of clones of polynucleotides in each library may vary. Further, since the probes used in claim 3 may be a chemical, an enzyme or an antibody, each of which may react with, and thus mark, different polynucleotides, the claimed genus comprises different species of libraries comprising different polynucleotides.

A description of a genus may be achieved by means of a recitation of a representative number of species, falling within the scope of the genus, or by means of a recitation of structural features common to the members of the genus, which features constitute a substantial portion of the genus. Regents of the University of California v. Eli Lilly & Co., 119 F3d 1559, 1569, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). In the instant case, however, the specification does not describe the structure (i.e. the sequences of each clone of a library) of any species, nor does it describe any structural feature (i.e. the sequence of each clone in a library) common to the members of the genus. No common structural attributes identify the members of the genus. While the specification gives example of how to make a library (see pages 113-116), it does not describe the structure of the library or libraries made. The general knowledge and level of skill in

RELATED PROCEEDINGS APPENDIX

As noted above on page 2 of this Brief on Appeal and pursuant to 37 C.F.R. § 41.37(c)(i) and (c)(x), Appellant has filed a Notice of Appeal in USSN 10/083,682, which is a CIP of the instant application. Inasmuch as no briefs have yet been filed, nor decisions received in that appeal, no documents are submitted with this Appendix.